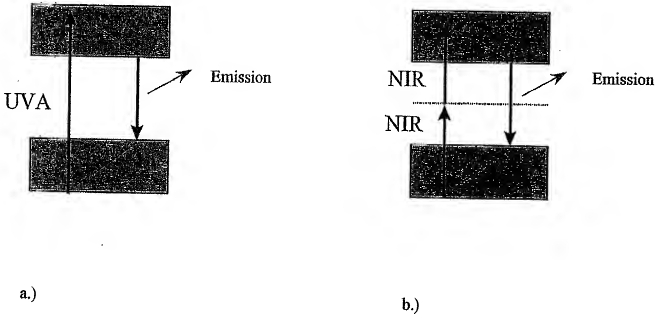


a.)

b.)

Figure: 1

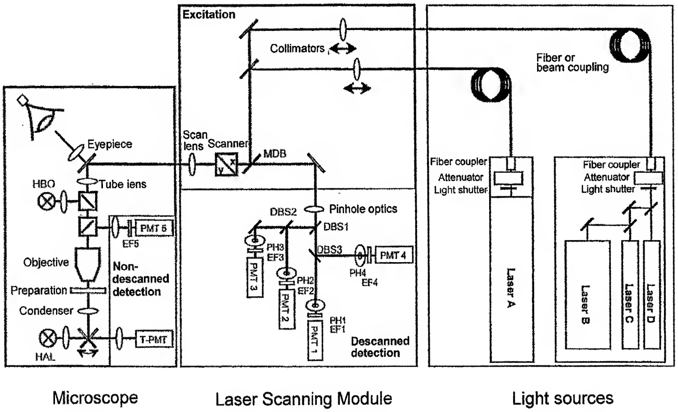

Excitation
Collimators
Fiber or
beam coupling
Scan
lens
Scanner
x
y
MDB
Eyepiece
Tube lens
HBO
PMT 5
EF5
Objective
Non-
descanned
detection
Preparation
Condenser
T-PMT
HAL
DBS2
Pinhole optics
DBS1
DBS3
PMT 4
PH4
EF4
PH3
EF3
PMT 3
PH2
EF2
PMT 2
PH1
EF1
PMT 1
Descanned
detection
Fiber coupler
Attenuator
Light shutter
Laser A
Fiber coupler
Attenuator
Light shutter
Laser B
Laser C
Laser D
Microscope
Laser Scanning Module
Light sources

Figure: 2

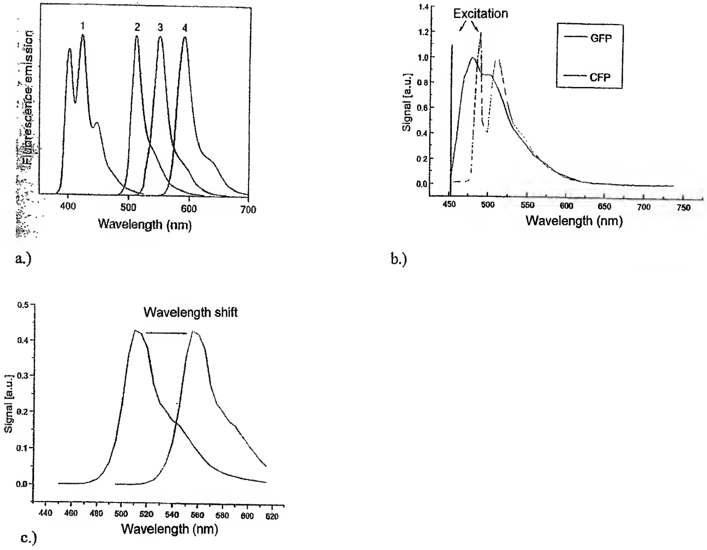
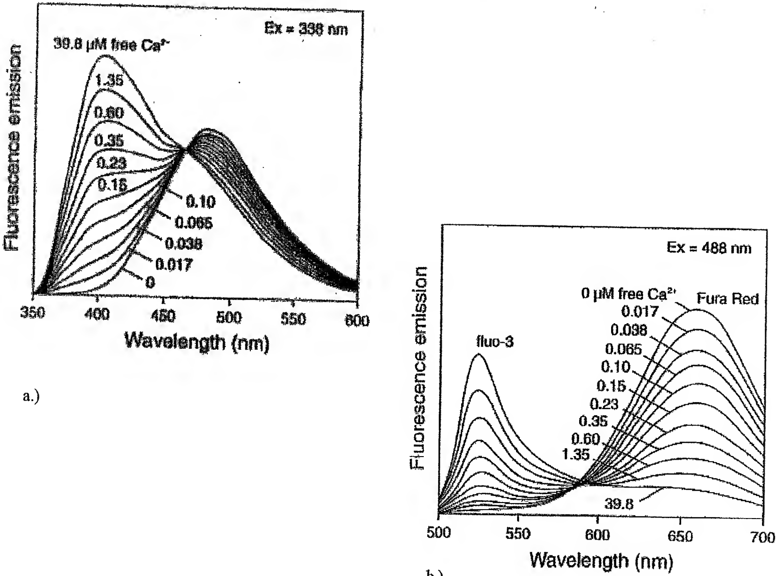

1
2
3
4
Fluorescence emission
400
500
600
700
Wavelength (nm)
a.)
Excitation
GFP
CFP
Signal [a.u.]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
450
500
550
600
650
700
750
Wavelength (nm)
b.)
Wavelength shift
Signal [a.u.]
0.0
0.1
0.2
0.3
0.4
0.5
440
460
480
500
520
540
560
580
600
620
Wavelength (nm)
c.)

Figure: 3

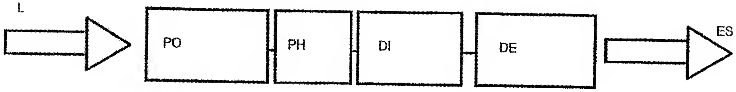

Ex = 338 nm
39.8 µM free Ca²⁺
1.35
0.60
0.35
0.23
0.15
0.10
0.065
0.038
0.017
0
Fluorescence emission
350
400
450
500
550
600
Wavelength (nm)
Ex = 488 nm
0 µM free Ca²⁺
Fura Red
0.017
0.038
0.065
0.10
0.15
0.23
0.35
0.60
1.35
39.8
fluo-3
Fluorescence emission
500
550
600
650
700
Wavelength (nm)

a.)

b.)

Figure: 4

Confocal detection



Nondescanned detection

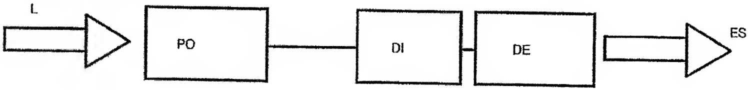


Figure: 5

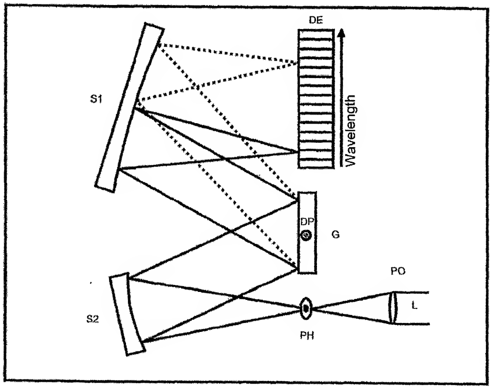


Figure: 6

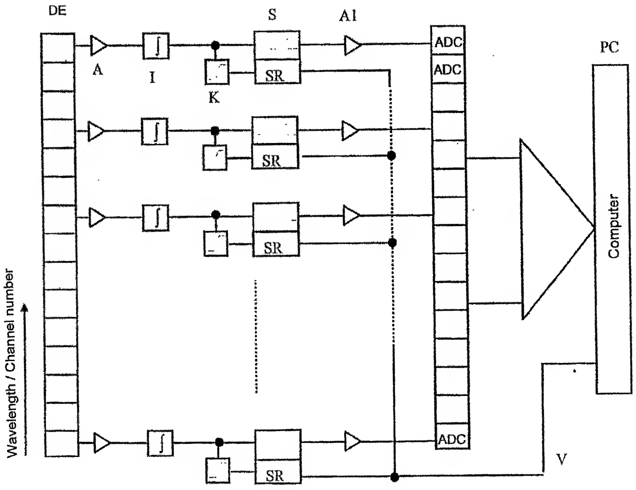

DE
A
I
K
S
SR
SR
SR
SR
A1
ADC
ADC
ADC
PC
Computer
V
Wavelength / Channel number

Figure: 7

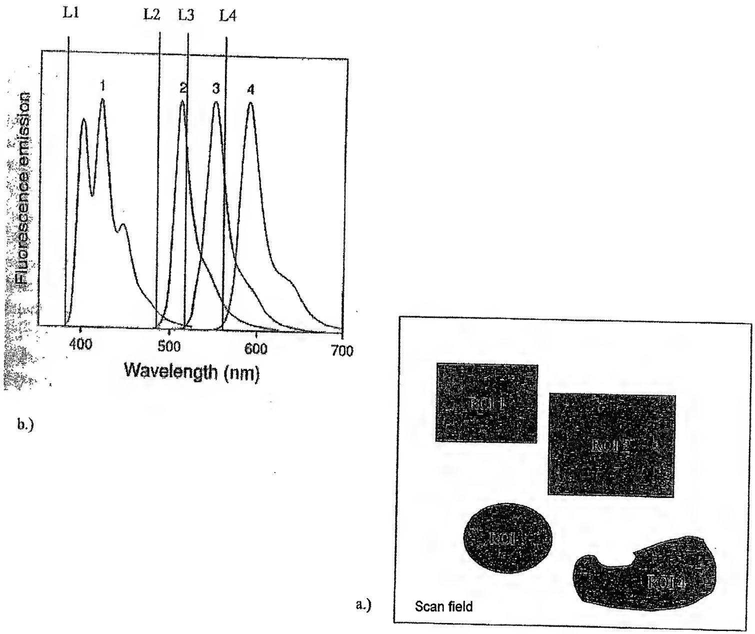
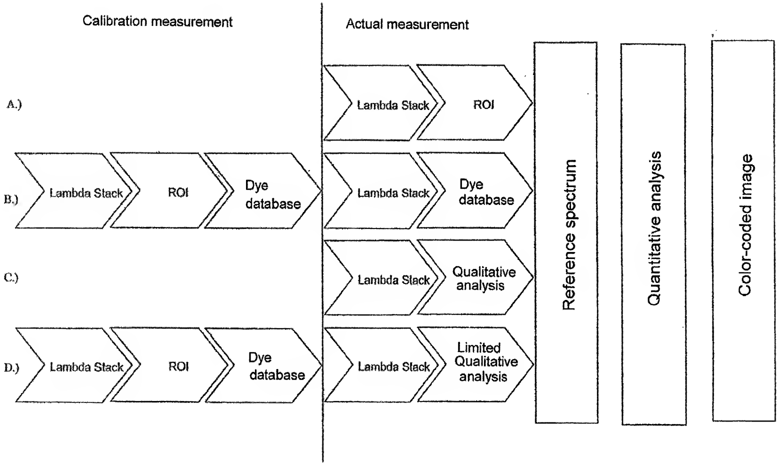

L1
L2
L3
L4
1
2
3
4
Fluorescence emission
400
500
600
700
Wavelength (nm)
b.)
a.)
ROI 1
ROI 2
ROI 3
ROI 4
Scan field

Figure: 8



Calibration measurement
Actual measurement
A.)
Lambda Stack
ROI
B.)
Lambda Stack
ROI
Dye database
Lambda Stack
Dye database
C.)
Lambda Stack
Qualitative analysis
D.)
Lambda Stack
ROI
Dye database
Lambda Stack
Limited Qualitative analysis
Reference spectrum
Quantitative analysis
Color-coded image

Figure: 9

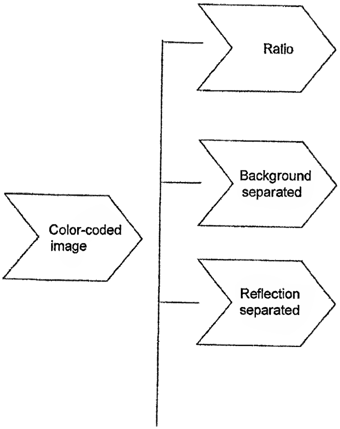

Color-coded
image
Ratio
Background
separated
Reflection
separated

Figure: 10

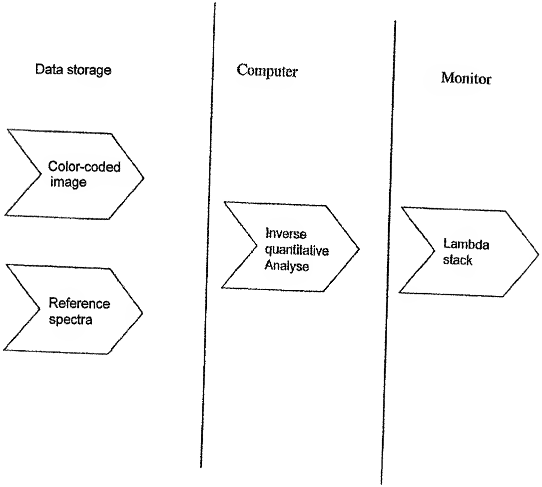
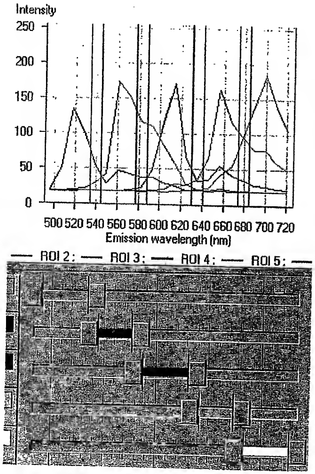
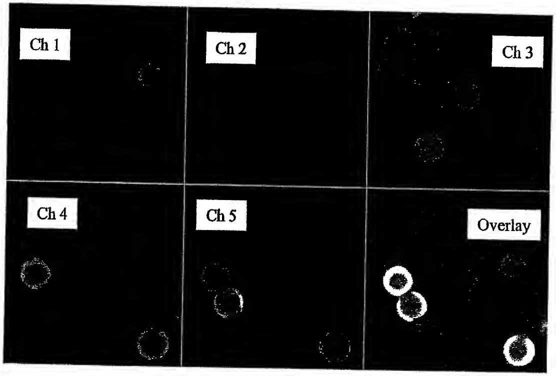

Data storage
Computer
Monitor
Color-coded image
Reference spectra
Inverse quantitative Analyse
Lambda stack

Figure: 11


Intensity
250
200
150
100
50
0
500 520 540 560 580 600 620 640 660 680 700 720
Emission wavelength (nm)
ROI 2:
ROI 3:
ROI 4:
ROI 5:

a.)


Ch 1
Ch 2
Ch 3
Ch 4
Ch 5
Overlay

b.)

Figure: 12

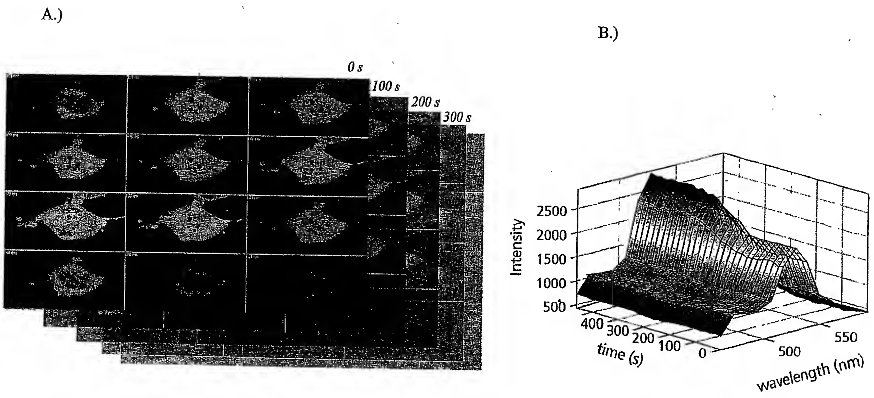

A.)
0 s
100 s
200 s
300 s
B.)
Intensity
2500
2000
1500
1000
500
time (s)
400
300
200
100
0
wavelength (nm)
500
550

Figure: 13

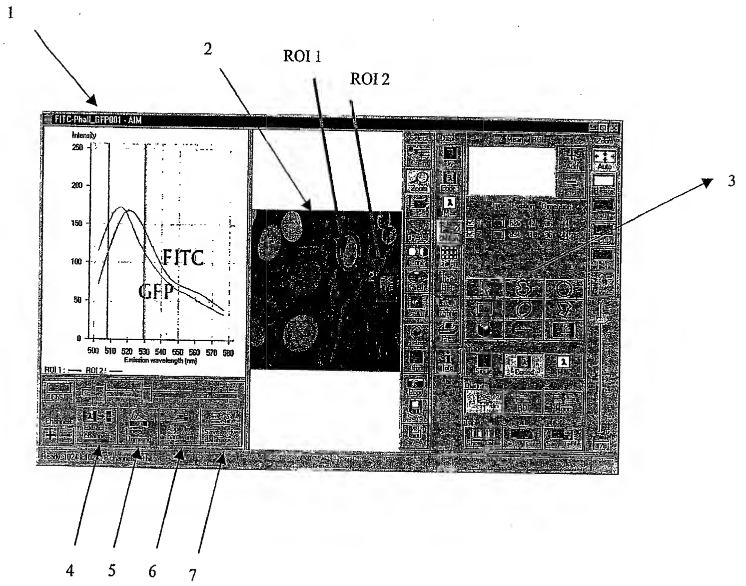

1
2
ROI 1
ROI 2
3
FITC-Phall_GFP001 - AIM
Intensity
250
200
150
100
50
0
500
510
520
530
540
550
560
570
580
Emission wavelength (nm)
FITC
GFP
ROI 1:
ROI 2:
Zoom
Auto
4
5
6
7

Figure: 14

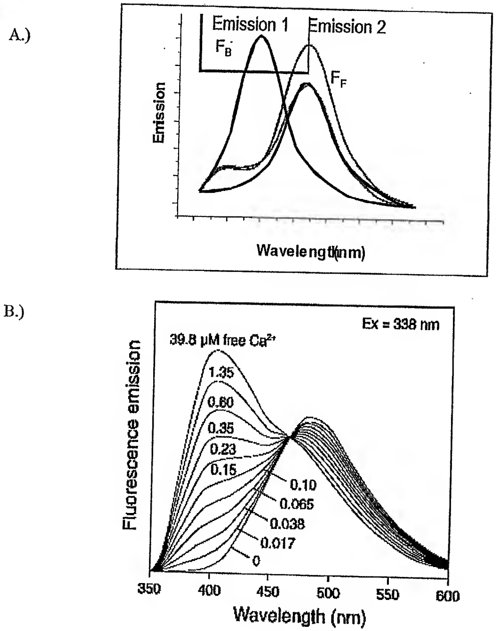

A.)
Emission 1
F_B
Emission 2
F_F
Emission
Wavelength (nm)
B.)
Ex = 338 nm
39.8 μM free Ca²⁺
1.35
0.60
0.35
0.23
0.15
0.10
0.065
0.038
0.017
0
Fluorescence emission
350
400
450
500
550
600
Wavelength (nm)

Figure: 15

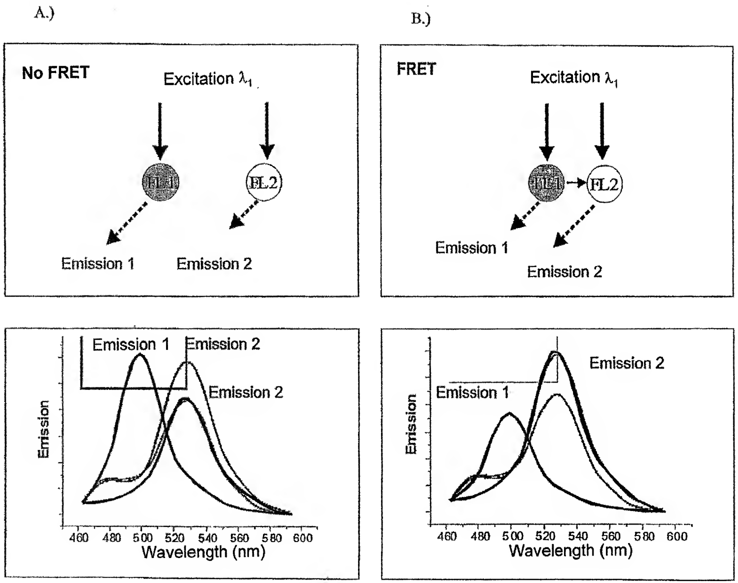
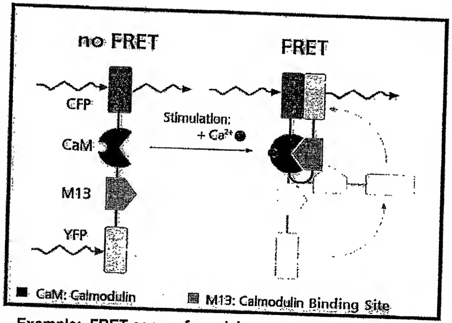

A.)
B.)
No FRET
Excitation λ_1
FL1
FL2
Emission 1
Emission 2
FRET
Excitation λ_1
FL1
FL2
Emission 1
Emission 2
Emission 1
Emission 2
Emission 2
Emission
460
480
500
520
540
560
580
600
Wavelength (nm)
Emission 1
Emission 2
Emission
460
480
500
520
540
560
580
600
Wavelength (nm)

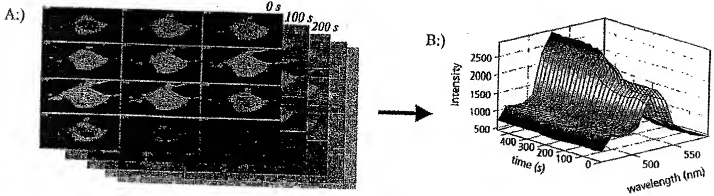
Figure: 16



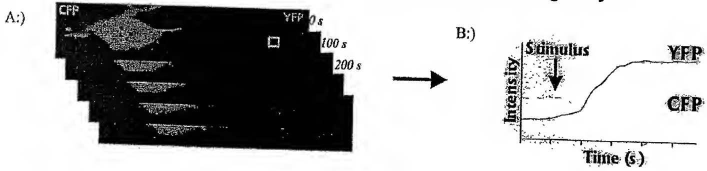
Example: FRET sensor for calcium concentration measurements
Yellow Cameleon2 (Miyawaki et al. Proc Natl Acad Sci USA 96, 2135-2140, (March 1999))

Figure: 17

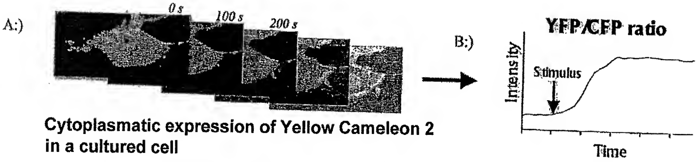
1. **Series of Lambda Stacks (xyλt)**



2. **Series of images with CFP and YFP signals after linear unmixing analysis**



3. **Series of ratio images (YFP/CFP)**



Cytoplasmatic expression of Yellow Cameleon 2 in a cultured cell

Figure: 18